

Applicant : Shih-Jen Liu, et al
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Attorney's Docket No.: 13886-002001 / 01P0325

REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. No new matter has been added.

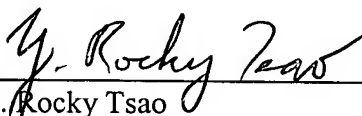
Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050, referencing attorney docket number 13886-002001.

Respectfully submitted,

Date: _____

6-5-02



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"Version With Markings to Show Changes Made"

In the specification:

Paragraph beginning at page 5, line 8, has been amended as follows:

_____Hsp70 gene was amplified from human hepatocellular carcinoma HepG2 cDNA with a gene-specific forward primer: 5'-cgcggatccATGGCCAAAGCCGCGGC-3' (SEQ ID NO:1), and a gene-specific reverse primer: 5'-cgcggatccCTAATCTACCTCCTCAATGG-3' (SEQ ID NO:2). The 1.92 kb Hsp DNA fragment was cleaved with *Bam*HI and ligated with a *Bam*HI-cleaved pRSETA vector. After transformation, plasmids were prepared from an overnight culture of transformed *E. coli* clones, and further analyzed by restriction enzyme digestion and sequencing. The recombinant plasmid containing Hsp70 gene was named as pRSETA/Hsp70.

Paragraph beginning at page 5, line 16, has been amended as follows:

Hsp C-terminal DNA fragment was amplified from pRSETA/Hsp70 with an HspC'-specific forward primer: 5'-gggaattcGCGATGCCAACGGCATCCTGAAC-3' (SEQ ID NO:3) and an HspC'-specific reverse primer: 5'-ggaaatttCTAATCTACCTCCTCAATGGTG-3' (SEQ ID NO:4). The 0.5 kb HspC' DNA fragment was cleaved with *Apo*I and ligated with an *Eco*RI-cleaved pRSET vector. After transformation, plasmids were prepared from an overnight culture of transformed *E. coli* clones, and further analyzed by restriction enzyme digestion and sequencing. The recombinant plasmid containing Hsp C-terminal DNA fragment was named as pRSET/HspC', which served as a backbone for construction of tumor antigen-HspC' expression plasmids.

Paragraph beginning at page 5, line 26, has been amended as follows:

HepG2 cells were homogenized in RNazolTMB solution, and total RNA was prepared according to the protocol provided with the kit. The cDNA was synthesized by SuperScriptTM II Reverse Transcriptase (GIBCO BRL) with an oligo-d(T)₁₂₋₁₈ primer. AFP gene was amplified from HepG2 cDNA with a gene-specific forward primer: 5'-gcggatccACACTGCATAGAAATG AATATG-3' (SEQ ID NO:5), and a gene-specific reverse primer: 5'-gcggatccAACTCCCAAAG CAGCACGAG-3' (SEQ ID NO:6). The 1.77 kb AFP DNA fragment was cleaved with *Bam*HI

and ligated with a *Bam*HI-cleaved pcDNA3 vector. After transformation, plasmids were prepared from an overnight culture of transformed *E. coli* clones, and further analyzed by restriction enzyme digestion and sequencing. The recombinant plasmid containing AFP gene was named as pcDNA3/AFP.

Paragraph beginning at page 6, line 17, has been amended as follows:

Total RNA was prepared from LNCaP cells with RNazolTMB (Tel-Test). LNCaP cDNA was synthesized by SuperScriptTM II Reverse Transcriptase (GIBCO BRL) with an oligo-d(T)₁₂₋₁₈ primer. PSA gene was amplified from LNCaP cDNA with a gene-specific forward primer: 5'-ATTGTGGGAGGCTGGGAGTG-3' (SEQ ID NO:7) and a gene-specific reverse primer: 5'-GGGGTTGGCCACGATGGTG-3' (SEQ ID NO:8). The PCR reaction was performed by DyNAzymeTM (FINNZYMES), and the 0.8 kb DNA fragment from PCR reaction was ligated to a pCRII vector (INVITROGEN) directly. After transformation, plasmids were prepared from an overnight culture of transformed *E. coli* clones, and further analyzed by restriction enzyme digestion and sequencing. The recombinant plasmid containing a sequence encoding the mature PSA was named as pCRII/mPSA.